PATENT SPECIFICATION 980,250

DRAWINGS ATTACHED.

980,250

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COMPLETE SPECIFICATION.

5-Cyclopropylhydantoins.

We, INNOTHERA, a Body Corporate, organized under the laws of France, of 10, Avenue Paul Vaillant Couturier, Arcueil, (Seine), France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to new 5-cyclopropylhydantoins.

The new compounds provided according to the invention have the general formula shown in Formula 1 of the accompanying drawings in which (a) R denotes the cyclopropyl or 2¹-thienyl group and R_1 denotes hydrogen or (b) R denotes the 2¹-thienyl group and R_1 denotes the methyl group. Thus the invention provides three new compounds, namely 5,5-dicyclopropylhydantoin, 5-cyclopropyl-5-(2¹-thienyl)-hydantoin and 3-methyl - 5 - cyclopropyl - 5 - (2¹ - thienyl)-hydantoin.

The three hydantoins have antispasmodic properties.

5,5-Dicyclopropylhydantoin and 5-cyclopropyl-5-(2¹-thienyl)-hydantoin may be made by reacting dicyclopropyl ketone or cyclopropyl 2-thienyl ketone respectively with ammonium carbonate and hydrogen cyanide or an alkali metal cyanide or ammonium cyanide. This reaction, using hydrogen cyanide, is indicated in Equation 1. It is preferred to use more than 1 mol of the carbonate and more than 1 mol of the cyanide per mole of the ketone. Preferably the carbonate and cyanide are together added to the ketone in two batches.

The 5,5-disubstituted hydantoins may be obtained in the following manner:

A solution of 0.1 mol of dicyclopropyl ketone or cyclopropyl 2-thienyl ketone in 250 ml of ethanol at 96° C. is mixed with a solution of 0.15 mol of potassium cyanide or other alkali metal cyanide and 0.3 mol of ammonium carbonate in 250 ml of water. The mixture is heated in an autoclave for a period of 18 hours at a temperature in the range 110°—120° C. The reaction mixture is cooled and 0.15 mol of potassium cyanide and 0.1 mol of ammonium carbonate are added to it and the reaction mixture is again maintained in the autoclave at a temperature in the range 110°—120° C. for a further 18 hours.

The cooled solution is concentrated to half-volume so as to eliminate the alcohol and is carefully acidified with hydrochloric acid to precipitate the 5,5-disubstituted hydantoin. The hydantoin separates out in solid form and can be centrifuged, washed, dried and recrystallised from an appropriate solvent such as ether, benzene, chloroform or ethanol.

3 - Methyl - 5 - cyclopropyl - 5 - (2¹-thienyl)-hydantoin is prepared by reacting 5-cyclopropyl-5-(2¹-thienyl)-hydantoin with dimethyl sulphate in the presence of sodium ethoxide or other alkali metal ethoxide in an absolute alcohol. The reaction is indicated in Equation 2 in which R_1 denotes the methyl group and R denotes the 2¹-thienyl group. The hydantoin reactant and dimethyl sulphate are preferably used in substantially equimolar amounts with refluxing of the mixture.

Thus the trisubstituted hydantoin may be produced in the following manner:—

0.1 mol of 5-cyclopropyl-5-(2¹-thienyl)-hydantoin is added to a solution of 0.1 mol of sodium in 450 ml of absolute ethanol, after which 0.1 mol of dimethyl sulphate is added. The mixture is heated under reflux until it becomes acid. The alcohol is then completely removed by distillation under

[*Price 4s. 6d.*]

reduced pressure. A few ml of water are added to the residue which is then dissolved in ether.

The ethereal solution is extracted with a 5% solution of sodium carbonate until acidification of the extraction phase no longer produces a precipitate. The ethereal phase is then dried over sodium sulphate and evaporated, leaving a residue of crude 3-methyl - 5 - cyclopropyl - 5 - (2^1 - thienyl)-hydantoin which is purified by recrystallisation from a suitable solvent, for example, ether, benzene, chloroform or ethanol.

The three new hydantoins are solid, crystalline compounds which are very stable and have well-defined melting points. They are of very low solubility in water but the two disubstituted hydantoins are soluble in solutions of the alkali metal carbonates and hydroxides whilst the trisubstituted hydantoin is insoluble in solutions of the alkali metal carbonates but soluble in solutions of the alkali metal hydroxides. The three hydantoins are soluble in many organic solvents, particularly ether, benzene, chloroform, methanol and ethanol.

The preparation of the three hydantoins is illustrated in the following examples.

EXAMPLE 1.

5,5-Dicyclopropylhydantoin
Empirical formula: $C_9H_{12}N_2O_2$
Molecular weight: 180.20

The structural formula of the compound is shown in Formula 2.

A solution of 4.5 grams of potassium cyanide and 13 grams of ammonium carbonate [$(NH_4)_2CO_3$, H_2O] was added to a solution of 5 grams of dicyclopropyl ketone in 100 ml of ethanol and the mixture was maintained at 110° C. in an autoclave for 18 hours. 4.5 grams of potassium cyanide and 4.4 grams of ammonium carbonate were then added to the autoclave and the whole mixture was maintained at 110° C. for a further period of 18 hours. The solution was then concentrated to half its volume, acidified with hydrochloric acid and the white precipitate of 5,5-dicyclopropylhydantoin obtained was centrifuged, washed with cold water, dried and purified by recrystallisation from ethanol.

White crystals, melting point 199° C. The yield was 73% of theory.

EXAMPLE 2.

5-Cyclopropyl-5-(2^1-thienyl)-hydantoin
Empirical formula: $C_{10}H_{10}N_2O_2S$
Molecular weight: 222.262

The structural formula of the compound is shown in Formula 3.

The hydantoin was prepared by the method described in Example 1, using 10 grams of cyclopropyl 2-thienyl ketone instead of the 5 grams of dicyclopropyl ketone. Upon recrystallisation from a chloroform-ethanol mixture, 5-cyclopropyl-5(2^1-thienyl)-hydantoin was obtained in the form of white crystals of melting point 200°—201° C. The yield was 50% of theory.

EXAMPLE 3.

3 - Methyl - 5 - cyclopropyl - 5 - (2^1-thienyl) - hydantoin
Empirical formula: $C_{11}H_{12}N_2O_2S$
Molecular weight: 236.22

The structural formula of the compound is shown in Formula 4.

3.48 grams of 5-cyclopropyl-5-(2^1-thienyl)-hydantoin and 1.96 grams of dimethyl sulphate were added to a solution of 0.36 gram of sodium in 80 ml of absolute ethanol and the mixture was boiled under reflux for 25 hours. The solvent was then removed by distillation and the residue, after adding to it 2 ml of water, was dissolved in 100 ml of ether. The ethereal phase was extracted several times with a 5% solution of sodium carbonate, dried over anhydrous sodium sulphate and the solvent was removed, leaving 3 - methyl - 5 - cyclopropyl - 5 - (2^1 - thienyl) - hydantoin. The hydantoin, upon recrystallisation from ether, was obtained in the form of white crystals, melting point 123°—124° C. The yield was 90% of theory.

The pharmacological properties of the new hydantoins in accordance with the invention can be appreciated from the tests indicated below:

I. — *Acute Toxicity.*

The hydantoins were placed in suspension in a 4% acacia syrup and were administered orally by means of an oesophageal probe to batches of female mice having an average weight of twenty grams ± one gram.

Dead animals were counted in the course of the twenty-four hours which followed ingestion.

The 50% lethal dose (DL 50) was calculated in accordance with the approximation method by establishing experimentally the regression line of the approximation percentages as a function of the logarithm of the dose administered.

The results obtained were as follows:

COMPOUNDS	DL 50±DL 50 in mg/Kg
3 - methyl - 5 - cyclopropyl-5 - (2^1 - thienyl) - hydantoin	1000±200
5 - cyclopropyl - 5 - (2^1-thienyl) - hydantoin	1000±188
5,5-dicyclopropyl-hydantoin	1200±71.6

II. — *Determination of Neurotoxicity.*

Neurotoxicity generally becomes apparent in mice in a plainly obvious form, for example, as somnolence, ebrious behaviour or agitation, the particular form depending on the toxic substance employed. At higher doses of the toxic substance, major disorders appear such as convulsions and paralysis.

A test found to be acceptable as an indication of the neurotoxicity of the hydantoins was the manifestation of ebrious behaviour within three hours of the oral administration of the hydantoins. The hydantoins were administered in three doses: 100, 200 and 300 mg/kg. An acknowledged neurotoxic dose is a dose which produces ebrious behaviour in at least 50% of the cases treated.

The results obtained are shown in the following table:

COMPOUNDS	50% neurotoxic doses in mg/Kg
5,5 - dicyclopropyl - hydantoin	<100
3 - methyl - 5 - cyclopropyl-5 - (2^1 - thienyl) - hydantoin	200
5 - cyclopropyl - 5 - (2^1-thienyl) - hydantoin	200

III. — *Extent of Protection Against Strychnine.*

Strychnine in an appropriate dose gives rise in animals to convulsive fits which can be inhibited by means of certain pharmacologically active substances. Tests were effected as follows:

1 mg of strychnine was administered to female mice per kilogram weight of the animal by intravenous injection. The strychnine was dissolved in physiological saline solution (0.9% sodium chloride), the quantities injected being 0.5 cc for 20 grams of body weight, while the rate of injection was constant and equal to 20″ per cc±1″.

The three hydantoins were in each case administered orally thirty minutes prior to the injection of strychnine. Since the intravenously-injected dose of 1 mg of strychnine per kilogram of body weight is always fatal in the case of mice, the animals killed within one half hour of the injection of strychnine were counted as a function of the dose administered. The linear regression between the calculation of the mortality percentage and the logarithm of the dose administered provides the effective dose in 50% of the cases treated (De 50). The results obtained are summarized in the following table:

COMPOUNDS	Effective 50% dose and its standard deviation in mg/Kg
3 - methyl - 5 - cyclopropyl-5 - (2^1 - thienyl) - hydantoin	105±27
5 - cyclopropyl - 5 - (2^1-thienyl) - hydantoin	175±47
5,5 - dicyclopropyl - hydantoin	250±65

IV. — *Protection Against Pentamethylene tetrazole.*

In much the same manner as strychnine, pentamethylene tetrazole produces convulsive fits which can be inhibited by certain substances.

The protective activity of the hydantoins was determined by a method of effective doses. A dose of 85 mg/kg of pentamethylene tetrazole was administered to batches of female mice by intraperitoneal injection (volume of the injection=0.1 cc, instantaneous rate). Such a dose results in fits in 100% of the mice and a 70% fatality rate. The fits are characterized by squeaking, rearing of the animal on its hind legs, convulsions, tonic spasms with extension of the rear paws which is generally followed by death. The animals were given the hydantoins orally, the dose administered being 100 mg/kg one hour prior to the injection of pentamethylene tetrazole. The mice which developed fits were isolated in order to prevent them from inducing fits in their neighbours. Those animals which had not developed fits within one half hour of the injection were then counted.

This test gave the following results:

COMPOUNDS	Percentage of animals protected by 100 mg/Kg of product administered per-orally
5 - cyclopropyl - 5 - (2^1-thienyl) - hydantoin	100
5,5 - dicyclopropyl - hydantoin	50
3 - methyl - 5 - cyclopropyl-5 - (2^1 - thienyl) - hydantoin	20

V. — *Extent of Protection Against the Attack of Electric Shock.*

Electric shocks produce under certain conditions the appearance of a tonic spasm of a convulsive fit, the spasm is characterized by the extension of the rear paws.

Electrodes for corneal stimulation and bathing of the eye with physiological saline solution (9 grams per litre). Square shock-sequence: duration 200 ms. 50 c/s, width 10 ms. Constant-voltage output.

(1) — The threshold voltage at which the appearance of the tonic spasm occurs was determined.

(2) — The hydantoins were administered orally in doses of 100, 50 and 25 mg/kg according to their activity.

(3) — One hour afterwards, the threshold voltage was re-applied; the ratio between the threshold value (A) prior ot administration of the hydantoin and the threshold value (B) after administration of the hydantoin was then caluculated. It is considered that the protection is significant when $\frac{A}{B} \leqslant 0.5$, that is, when the new threshold is at least double the original threshold.

The results obtained are summarized in the following table:

COMPOUNDS	Percentage of animals protected in accordance with the dose in mg/Kg			
	100	50	25	12.5
5 - cyclopropyl - 5 - (2¹-thienyl - hydantoin	100	100	70	0
3 - methyl - 5 - cyclopropyl-5 - (2¹ - thienyl) - hydantoin	100	—	—	—
5,5 - dicyclopropyl - hydantoin	80	—	—	—

(—) doses not tested.

VI. — *Determination of the Protection in the Case of an Increase by 50% of the Threshold of Minimum Attack of Electric Shock.*

The operation was carried out in the following manner:

The type of attack sought in this case entailed all the minor disorders which result from an electric shock. The minimum attack began with trembling of the jaw followed by a phase of hebetude (animal in a motionless state), then by the appearance in a large number of cases of a phase of hyperactivity with running and jumping.

The most characteristic phase was the hebetude phase, and this phase was used as a basis for determining the presence or absence of attack.

The method of stimulation was identical with that used for the maximum attack but in this case there was employed a "constant current" output.

The threshold of attack was predetermined in the case of each animal several days before carrying out the experiment; when this threshold remained constant for two consecutive days, the hydantoin to be tested was administered orally on the third day to batches of female mice, the dose administered being 100 mg/kg. One hour afterwards, the animal was subjected to an electric shock having a current intensity which was 50% higher than that of the previously determined threshold shock. When this shock was ineffective, the animal was considered to be protected.

These results are summarized in the table given hereunder:

COMPOUNDS	Percentage of animals protected in the case of the threshold shock value by 50% (dose of 100 mg/Kg)
5 - cyclopropyl - 5 - (2¹-thienyl) - hydantoin	100
3 - methyl - 5 - cyclopropyl-5 - (2¹ - thienyl) - hydantoin	14
5,5 - dicyclopropyl - hydantoin	12

WHAT WE CLAIM IS: —

1. 5,5-Dicyclopropylhydantoin.
2. 5-Cyclopropyl-5-(2¹-thienyl)-hydantoin.
3. 3 - Methyl - 5 - cyclopropyl - 5 - (2¹-thienyl)-hydantoin.
4. A method for the production of 5,5-dicyclopropylhydantoin, which comprises reacting dicyclopropyl ketone with ammonium carbonate and hydrogen cyanide or an alkali metal cyanide or ammonium cyanide.
5. A method for the production of 5-cyclopropyl-5-(2¹-thienyl)-hydantoin, which comprises reacting cyclopropyl 2-thienyl ketone with ammonium carbonate and hydrogen cyanide or an alkali metal cyanide or ammonium cyanide.
6. A method according to Claim 4 or Claim 5, in which the carbonate and cyanide are together added to the ketone in two batches.
7. A method according to any one of Claims 4 to 6, in which more than 1 mol of the carbonate and more than 1 mol of the cyanide are used per mol of the ketone.
8. A method for the production of 3-methyl - 5 - cyclopropyl - 5 - (2¹ - thienyl)-

hydantoin, which comprises reacting 5-cyclopropyl-5-(2¹-thienyl)-hydantoin with dimethyl sulphate in the presence of an alkali metal ethoxide in an absolute alcohol.

9. A method according to Claim 8, in which the hydantoin reactant and dimethyl sulphate are used in substantially equimolar amounts and the reaction mixture is refluxed.

10. A method for the production of 5,5-dicyclopropylhydantoin, substantially as hereinbefore described with reference to Example 1.

11. A method for the production of 5-cyclopropyl-5-(2¹-thienyl)-hydantoin, substantially as hereinbefore described with reference to Example 2.

12. A method for the production of 3-methyl - 5 - cyclopropyl - 5 - (2¹ - thienyl)-hydantoin, substantially as hereinbefore described with reference to Example 3.

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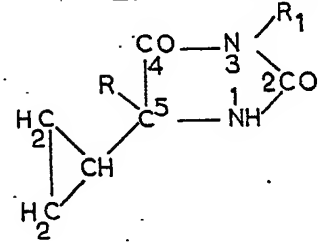
Formula 1



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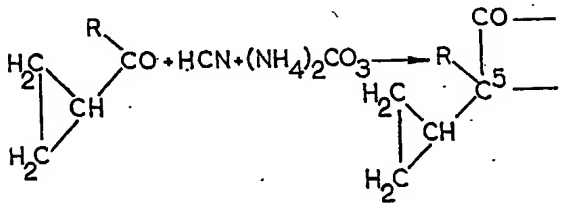

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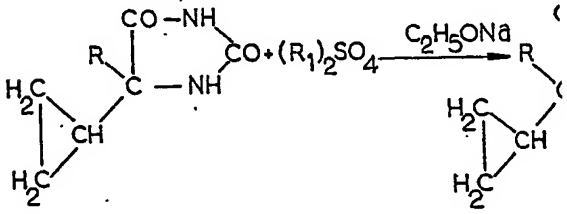

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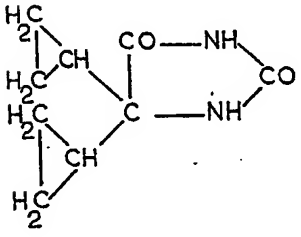

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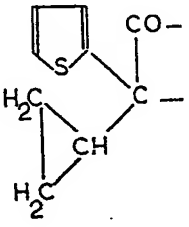

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2. SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheets 1 & 2

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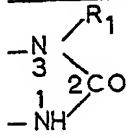

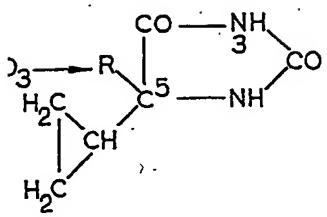

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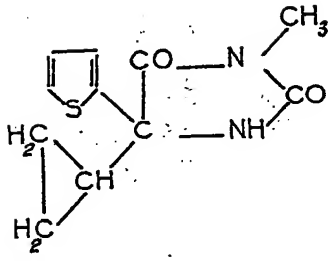

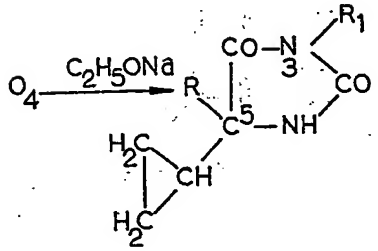

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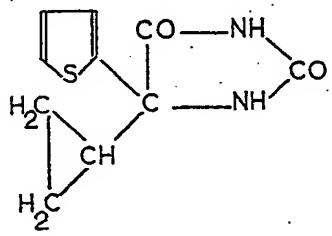

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Sheets 1 & 2

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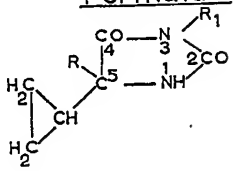

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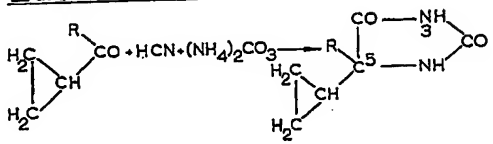

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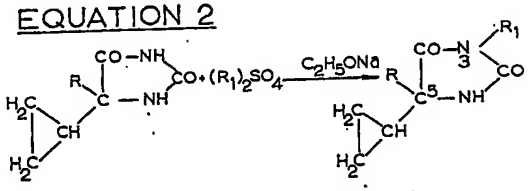

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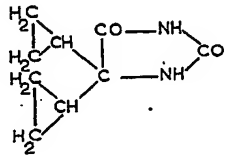

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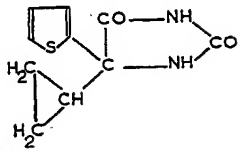

Formula 4